REMARKS/ARGUMENTS

Status of the Claims

With this amendment, claims 2 and 13-16 are pending and are under examination in this application.

Claim 2 has been amended for consistency and not in response to a rejection or for any other reason related to patentability. Support for this amendment may be found, for example, on page 3, paragraphs [0041] and [0042] of U.S. Patent Application Publication US 2006/0183225 (hereafter the '225 Publication"), which is the publication of the '664 Application. Claim 3 has been amended to relate the phrase "histone deacetylase inhibitor" to its antecedent basis by inserting the word "the" before the phrase. Claim 3 also has been amended to depend from claim 2 rather than claim 1.

Claims 5 and 14 were amended to delete "treatment" as this word does not appear in the claim from which each of these claims depends and claim 13 was amended to depend from claim 3 and thus to provide antecedent basis for the words "contact" and "contact time" in claim 14.

Claim 16 has been amended to correct an inadvertent typographical error. Specifically, claim 16 has been amended to recite the word "chromatin" rather than "chromatic". Support for this amendment may be found, for example, in originally filed claim 2. Claim 16 also has been broadened. Specifically, step ii) has been deleted and steps iii), iv), and v) have been re-numbered as steps ii), iii), and iv), respectively. In claim 16, the recitation of both deleted step ii) and former step iii) (now current step ii)) was not required because former step iii (now current step ii)) necessarily included deleted step ii). Thus, former step ii) has been deleted and former step iii) (now current step ii)) remains in addition to former steps iv) and v) (now current steps iii) and iv)). Support for this amendment can be found, for example, in claim 2 as originally filed and in the '225 Publication, paragraph [0078]. Lastly, claim 16 has been amended for greater clarity by adding the word "cells" after the phrase "in which" to make it easier for the reader to understand the reference.

Applicants expressly reserve the right to pursue any cancelled subject matter in subsequent applications claiming benefit from this application.

No new matter has been added.

December 3, 2008 Interview

Applicants thank Examiners Woitach and Leavitt for the courtesy of an interview on December 3, 2008 with Drs. Hidetaka Seo (one of the named inventors of the current application), Masa Fujiwara, and Shoko Yoshida (members of the licensee of Applicant's assignee) and Applicants' representatives, Adda C. Gogoris (Reg. No. 29,714) and Dr. Shilpa V. Patel (Reg. No. 57,983). In the interview, the rejections were discussed and the Examiners considered Applicants' proposal to overcome the rejections set forth in the July 25, 2008 Office Action.

Applicants and their representatives deeply appreciate the Examiners' courtesy and the thorough preparation and careful attention accorded by both Examiners to this application and to the Applicants' position.

The present response, submitted in accordance with the above-mentioned interview of December 3, 2008, is intended to be fully responsive to all points of rejection and is believed to place the application in condition for allowance. Favorable reconsideration and allowance of the present application are hereby respectfully requested.

The Declaration of Dr. Hidetaka Seo, Ph.D.

Pursuant to 37 C.F.R. § 1.132, a Declaration by Dr. Dr. Hidetaka Seo, Ph.D. ("the Seo Declaration"), a named co-inventor, accompanies this Amendment and is referred to in the following comments.

Information Disclosure Statement

Applicants submit herewith an IDS for references relied upon in the Seo Declaration and the following comments, even though the same are employed in an evidentiary capacity. It is respectfully requested that the Examiner enter and consider the cited references, and that these references be listed on the face of any patent issuing from the current application.

Rejections under 35 U.S.C. § 103, Obviousness

Claims 2 and 15 stand rejected under 35 U.S.C. §103(a) as allegedly being obvious over Sonoda et al., *Phi. Trans. R. Soc.*, 2001, London, 2001, 11-117 (hereafter "Sonoda"), in view of McMurry et al, *Science*, 2000, 495-498 (hereafter "McMurry") and further in view of Watson, et al., *Recombinant DNA*, 2001, pp. 297-304 (hereafter "Watson").

Prior to the present response, the Examiner's position was that Sonoda teaches the use of the chicken B-lymphocyte line DT40, of which homologous recombination is an intrinsic characteristic, for the production of diverse antibodies. The Examiner also states that the teachings of Sonoda indicate that the B-lymphocyte line DT40 undergoes rearrangement of antibody chains by homologous recombination of the chicken Ig loci to generate diversity. However, the Examiner acknowledges that Sonoda does not specifically teach enhancing homologous recombination by relaxing the chromatin.

The Examiner relies upon McMurry to supply the missing teaching of Sonoda. According to the Examiner, McMurry provides a role for histone hyperacetylation in the developmental regulation of V(D)J recombination. The Examiner acknowledges that Sonoda and McMurry do not specifically disclose that regulation of V(D)J recombination is the same in T cells and immunocytes, but relies upon Watson as disclosing that recombination of the V, D, J and C segments in T cell receptor genes is similar to the recombination of the antibody V(D)J genes. The Examiner concludes that a skilled artisan would have found it obvious to improve the production of antibodies by homologous recombination by making DNA more accessible to recombinant enzymes to regulate V(D)J recombination.

The Applicants asserted that the skilled artisan would not have looked to the teachings of Watson or McMurry, for guidance to arrive at the claimed methods because V(D)J recombination (in both B cells and T cells) and homologous recombination (in the gene conversion method of the present invention) are different mechanisms. V(D)J recombination requires a "site-specific endonuclease", called RAG1-RAG2, which triggers DNA break formation by a mechanism similar to the mechanism of transposition. On the other hand, the mechanism of gene conversion

(homologous recombination) requires activation-induced cytidine deaminase (AID). The Examiner, citing to Martin and Scharff (*Nature Reviews*, 2002, Vol.2, pgs. 605-614; hereafter "Martin"), acknowledged that RAG is functional only in V(D)J recombination, and has a different mechanism of action than AID that is active in gene conversion. However, in the July 25, 2008 Office Action, the Examiner took the position that

> both recombinant enzymes require accessibility to the double stranded DNA to induce DNA breaks. Therefore, relaxing the chromosomal DNA using deacetylase inhibitors should be reasonably expected to improve AID mediated recombination for the same reason it improves RAG mediated V(D)J recombination by facilitating access of both recombinant enzymes to the chromosomal DNA.

See July 25, 2008 Office Action, page 6. The grounds for rejection are not well taken and are respectfully traversed.

During the December 3rd interview, Applicants presented four points that were directed principally to the inadequacy of the prima facie case of obviousness, and that are discussed in detail below:

1. The processes of V(D)J recombination and homologous recombination are different, and hence teachings applicable to one cannot be willy nilly applied to the other;
2. McMurry, which observes that histone acetylation is beneficial in V(D)J recombination, is limited to V(D)J recombination and is not applicable to homologous recombination;
3. The mechanism of action of gene conversion (of which homologous recombination is a species) was not elucidated at the relevant time[1], and specifically the mechanism of AID was unknown; and
4. Regardless of the mechanism of AID, the effect of trichostatin A (TSA) in cells undergoing gene conversion (*i.e.*, whether TSA activated or inactivated homologous recombination) was unpredictable at the relevant time.

[1] The relevant time is the filing of the priority application, Japanese Application No. JP 2002-221232.

The Processes of V(D)J Recombination and Homologous Recombination are Different

In chicken-derived B cells, homologous recombination involves gene conversion. *See* Seo Declaration, ¶7. At the relevant time, it was known in the field that the processes of V(D)J recombination and homologous recombination are very different. *See* Seo Declaration, ¶¶8-14. Important differences between the processes that are discussed in ¶¶8-14 of the Seo Declaration are highlighted in Table 1 below.

Table 1: Highlighted Differences between Homologous Recombination and V(D(J) Recombination.

Homologous Recombination	**V(D(J) Recombination (nonhomologous DNA end joining)**
Double-strand breaks are repaired during **late S and G2 phases** of the cell cycle. *See* Seo Declaration, ¶9.	Double-stranded breaks are repaired during **G0, G1, and early S phases** of the cell cycle. *See* Seo Declaration, ¶9.
Initiation of recombination process occurs in **mature lymphocytes** in a **non-sequence specific-manner**. *See* Seo Declaration, ¶¶10-11.	Initiation of recombination process occurs in **developing lymphocytes and only between Ig and T cell receptor gene segments** in a **sequence specific-manner**. *See* Seo Declaration, ¶¶10-11.
Full repertoire of DNA is accessible because **DNA loci are not deleted** thereby permitting repeated and continuous recombination to **achieve maximal generation of antibody diversity.** *See* Seo Declaration, ¶12.	**Less than the full repertoire of DNA** is accessible because **genomic DNA undergoes deletion** thereby **limiting the diversity of antibodies** that may be generated. *See* Seo Declaration, ¶¶13-14.

From the above table, it is evident that because each process is different, cells undergoing homologous recombination would not also be undergoing V(D)J recombination. *See* Seo Declaration, ¶9. Thus, for at least one of the above differences, a skilled worker would not have relied upon references discussing V(D)J recombination for guidance to enhance DNA homologous recombination as called for by the present claims because teachings applicable to V(D)J recombination are not applicable to homologous recombination. *See* Seo Declaration, ¶15.

In rebuttal of the Examiner's position that accessibility of DNA would enhance both V(D)J recombination and homologous recombination, Applicants submit that this was not predictable, especially as of the effective filing date of the present application for the following reasons:

McMurry Would Not Have Led a Skilled Worker to Use a Histone Deacetylase Inhibitor to Enhance Homologous Recombination

A skilled worker would not have been motivated by McMurry, alone or in combination with the other cited prior art, because the skilled worker would also be familiar with Agata et al. (*J. Exp. Med.*, 2001, 193(7): 873-879; hereafter referred to as "Agata")[2], which elucidates why the teachings of McMurry are inapplicable to designing the claimed methods. While McMurry teaches that histone acetylation at the T cell receptor locus is enhanced, it does not provide a basis for a reasonable expectation of success for enhanced homologous recombination, nor does it teach using a histone deacetylase inhibitor. *See* Seo Declaration, ¶16.

In Agata, TSA activated V(D)J recombination at loci that are naturally silent and inactive and TSA had little effect at loci that are normally active. *See* Seo Declaration, ¶17. A skilled worker would have known that homologous recombination requires V gene loci that are already active. The same skilled worker having read Agata and McMurry would not have had a reasonable expectation of success to use a histone deacetylase inhibitor to promote gene conversion because TSA in Agata had very little effect on active V gene loci. Therefore, the findings of Agata establish why a skilled worker would not have had a reasonable expectation that hyperacetylation, as

[2] Agata was filed by Applicants in an IDS dated January 26, 2005, and was made of record by Examiner Leavitt on January 23, 2008.

McMurry teaches for V(D)J, would be beneficial in the gene conversion (homologous recombination) context of the present invention. *See* Seo Declaration, ¶18.

The Mechanism of AID in Gene Conversion was Unknown at the Relevant Time

At the relevant time, the mechanism of gene conversion was not elucidated. Specifically, the mechanism of action for AID, which is required by gene conversion, was unknown. *See* Seo Declaration, ¶19. In fact, Martin, which the Examiner cited, states that "there is still considerable uncertainty about the mechanism of action of AID". *See, e.g.*, Martin, page 605, last sentence of the abstract.

At the relevant time, there were two principle hypotheses being advanced: the DNA editing model and the RNA editing model; however, which hypothesis, if either, was in operation in gene conversion (homologous recombination) was unknown. *See* Seo Declaration, ¶20. The first hypothesis, the DNA editing model, would have AID initiate gene conversion directly by catalyzing cytosine deaminase resulting in guanine/uracil (G/U) mismatches. *See* Seo Declaration, ¶21. On the other hand, the second hypothesis, the RNA editing model, would have AID modify, for example, mRNA for genes involved in gene conversion leading to the translation of an active protein that then introduces a DNA alteration in the rearranged V gene segment. *See* Seo Declaration, ¶22. A skilled worker at the relevant time would not have known in gene conversion, if AID modified DNA directly or indirectly, and so the skilled worker would not have been able to reasonably predict that V(D)J recombination teachings were applicable to gene conversion (homologous recombination). *See* Seo Declaration, ¶19-22.

As we explain below, a skilled worker would not have looked to the teachings of prior art cited by the Examiner for guidance to arrive at the claimed methods because it was unknown whether AID initiated gene conversion by modifying DNA or RNA. **The existence of at least two hypotheses already would have injected uncertainty in the outcome of adding a reagent such as a histone deacetylase inhibitor as discussed in the comments immediately below.**

Regardless of the Mechanism of AID, the Effect of TSA in Cells Undergoing Gene Conversion Was Unpredictable at the Relevant Time

As discussed above, the mechanism of AID was unknown. Furthermore, either hypothesis advanced did not raise the expectation that TSA would affect gene conversion. In fact, the effect of TSA on gene conversion was unpredictable because for each known hypothesis, TSA would have introduced further uncertainty. *See* Seo Declaration, ¶23.

Moreover, whatever the mechanism of action of AID, there were reasons why a skilled worker would not have reasonably expected addition of a histone deacetylase inhibitor to increase recombination events. With each, the RNA editing model or the DNA editing model, it would not have been reasonably predictable by a skilled artisan what effect TSA would have on gene conversion.

With respect to the DNA editing model, one could say that it was plausible that a histone deactylase inhibitor could have resulted in accelerated DNA deamination followed by enhanced gene conversion frequency. On the other hand, it would then be also plausible with the DNA editing model that a histone deacetylase inhibitor could have had the undesirable results of up-regulation of inhibitors of AID and down-regulation of homologous recombination factors. *See* Seo Declaration, ¶24. A skilled worker would not have been able to reasonably predict how or which of the numerous proteins involved in homologous recombination would have been affected by the up-regulation of inhibitors of AID and down-regulation of homologous recombination factors.

With respect to the RNA editing model, a histone deacetylase inhibitor might have affected RNA, not DNA directly, thereby it could have altered expression of DNA-modifying enzymes negatively or positively. A skilled worker would not have been able to reasonably predict which of the numerous enzymes involved in homologous recombination would have been upregulated or downregulated. Introducing further uncertainty, the altered expression of enzymes involved in homologous recombination could have enhanced or impaired gene conversion. *See* Seo Declaration, ¶25. In fact, the skilled artisan, at the relevant time would not have had a reasonable expectation of success to combine the elements referred to by the Examiner in the cited prior art because it was unpredictable whether or how a histone deacetylase inhibitor would have altered the

expression pattern of DNA-modifying enzymes and whether or how the modified expression would impact gene conversion, a process that proceeds in the present claims via homologous recombination. *Id.* *See* MPEP §2143.01(III) ("The mere fact that references can be combined or modified does not render the resultant combination obvious unless the results would have been predictable to one of ordinary skill in the art.") (emphasis in original).

Therefore, determining the various elements that can be combined to design a method suitable for diverse antibody production using DNA homologous recombination by relaxing with a histone deacetylase inhibitor the chromatin structure of chromosomes requires experimentation and is unpredictable until a method of that type is designed and used. In fact, it was only through the experiments carried out by the current inventors as described in the specification that it became clear that a histone deacetylase inhibitor could relax the chromatin structure in gene conversion to create a population of diverse antibodies as called for by the pending claims. *See* MPEP § 2145(X)(B). *See KSR Int'l Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1740 (2007) ("a court must ask whether the improvement is more than the predictable use of prior art elements according to their established functions").

Specifically, the inventors of the current application discovered that treatment of chicken-derived B cells with TSA led to an extreme increase (e.g., at least 2 orders of magnitude) in homologous recombination. *See* Seo Declaration, ¶26. The results, from the experiments carried out by the inventors of the current application, provide proof that it was unpredictable that TSA could be used to enhance homologous recombination in chicken-derived B cells especially given that the mechanism of AID in gene conversion at the relevant date was unknown. Furthermore, the results demonstrate that the teachings of V(D)J recombination, as exemplified by McMurry and Agata, are not applicable to the claimed methods relating to homologous recombination. A skilled worker would not have looked to V(D)J recombination teachings for guidance to generate a diversity of antibodies using homologous recombination because, for example, in Agata, only a mild increase of several percent in V(D)J recombination events was observed. *See* Seo Declaration, ¶14. Thus at the relevant time, a skilled worker having read the prior art cited by the Examiner would not have been led to design the claimed methods.

For at least the reasons set forth above, pending claims 2 and 15 are not obvious over the prior art of record. Accordingly, it is respectfully requested that the Examiner reconsider and withdraw this rejection.

* * * * *

Claims 13-14 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Sonoda, McMurry, Watson, and further in view of Choy et al. (2002, *Mol Cell Biol.*, 8215-8225; hereafter "Choy"). The Examiner relies upon Sonoda, McMurry, and Watson for the same reasons that claims 2 and 15 have been rejected, and additionally relies upon Choy as teaching that transcription requires acetylation of histone N-terminal tails to promote an open chromatin conformation during chromosomal replication and as teaching restoration of H4 acetylation with TSA. According to the Examiner, it would have been *prima facie* obvious for a skilled worker use TSA based upon the teachings of Choy to promote an open chromatin conformation. The ground for rejection is not well taken and is respectfully traversed.

Claim 13 depends from claim 1 and specifies that the inhibitor is TSA. Claim 14 depends from claim 13 and species the concentration of TSA. However, Choy does not cure the deficiencies of Sonoda, McMurry, and Watson, as discussed in the foregoing comments. In fact, nothing in Choy, alone or in combination with the other cited prior art references, describes or suggests the claimed methods for antibody production. Choy teaches transcription of a gene. One of ordinary skill in the art, when looking to design a method of enhancing homologous recombination during antibody production, would not seek guidance from a reference relating to a non-analogous art, *i.e.*, gene transcription. Thus, a skilled worker would not look to Choy for such guidance. Accordingly, it is respectfully requested that the Examiner reconsider and withdraw this rejection, as well.

* * * * *

Claim 16 is rejected under 35 U.S.C. § 103(a) as being unpatentable over Sonoda, McMurry, Watson, and further in view of Sale et al., U.S. Patent 7,122,339 (hereafter "Sale"). The Examiner relies upon Sonoda, McMurry, and Watson for the same reasons that claims 2 and 15 have been rejected, and additionally relies upon Sale as teaching a method for selection of antibodies from a

pool of generated antibodies that are expressed on the surface of a host cell and as teaching that it is advantageous to culture and establish such selected clonal populations. The ground for rejection is not well taken and is respectfully traversed.

Claim 16 is directed to a method for producing an antibody which can bind to a target antigen comprising the steps of 1) enhancing DNA homologous recombination at an antibody locus by relaxation of the chromatin structure at the antibody locus using a histone deacetylase inhibitor whereby diverse immunocytes are obtained; 2) contacting the immunocytes with the target antigen; 3) selecting an immunocyte producing antibody that can bind the target antigen; and culturing the selected immunocyte. However, Sale does not cure the deficiencies of Sonoda, McMurry, and Watson, as discussed in the foregoing comments. In fact, nothing in Sale, alone or in combination with the other cited prior art references, describes or suggests the claimed method. Sale teaches a method for preparing an antibody-producing cell line capable of directed constitutive hypermutation of a specific nucleic acid region. One of ordinary skill in the art, when looking to design a method of enhancing homologous recombination during antibody production, would not seek guidance from a method for preparing an antibody producing cell line as taught by Sale. Accordingly, it is respectfully requested that the Examiner reconsider and withdraw this rejection, as well.

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For at least the reasons set forth above, pending claims 2 and 13-16 are not obvious over the cited prior art. Accordingly, it is respectfully requested that the Examiner reconsider and withdraw these rejections.

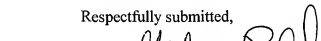
CONCLUSION

In view of the above amendment and remarks, it is respectfully requested that the application be reconsidered and that all pending claims be allowed and the case passed to issue. Applicants reserve the right to pursue the cancelled and/or non-elected subject matter in one or more continuation or divisional applications.

If there are any other issues remaining that the Examiner believes can be resolved through either a Supplemental Response or an Examiner's Amendment, the Examiner is respectfully requested to contact the undersigned at the telephone number indicated below.

Dated: June 22, 2009

Respectfully submitted,

By 

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